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PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC LEAF EXTRACT OF *DRYPETES SEPIARIA* (WIGHT & ARN.) PAX. & HOFFIM. FROM SILAMBUR SACRED GROVE, TAMILNADU

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ABSTRACT

Drypetes sepiaria (Wight & Arn.) Pax. & Hoffim a medium sized tree member of Euphorbiaceae was investigated to determine the phytochemical constituents present in various extracts of the leaves through GC-MS analysis. Powdered leaf plant materials were subjected to successive extraction with organic solvents such as methanol by Soxhlet extraction method. In the present study, GC-MS analysis revealed that a total of 23 different compounds identified by using methanol extract and all the identified compounds were medicinally valuable for the treatment of various human ailments. In addition, all the phytochemical compounds were needed for further investigations on toxicological aspects for the development of new lead of therapeutic interest.

Keywords: *Drypetes sepiaria* preliminary screening, GC-MS analysis.

1. INTRODUCTION

Plants have been a rich source for drug discovery (Mishra and Tiwari, 2011). Plants and plant parts have been provide a good source of pharmaceutical active compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, saponin and some other secondary metabolites, which are rich in valuable bioactivities of antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic (Maridass *et al.,* 2008). The genus *Drypetes* (Putranjivaceae (Euphorbiaceae) comprises nearly 160 species which has been used in the folk medicine of many cultures for many years (Nganga *et al.,* 2008). Even though the species was different, they used to treat similar disorders. Among the members of the genus *Drypetes* earlier phytochemical studies on some species including *D. parrifolia, D. laciniata, D. inaequalis, D. armoracia, D. gossweileri, D. molunduana, D. roxburghii* have yielded flavonoids, chalcone glycosides, saponins, tripterpenoids, phenolics, alkaloids, etc.

Drypetes sepiaria (Wight & Arn.) Pax. & Hoffim. an ever green tree locally known as Kalvirai (Tamil) commonly grown in foothills and shrub jungles and some sacred groves of Tamil Nadu. *Drypetes sepiaria* is traditionally used to treat pain and inflammation and seeds are used as a wild edible food and their root paste can be used as an antidote for scorpion bite. Decoction of leaves and seeds is also noted for reducing rheumatic inflammation (Arinathan *et al.,* 2007; Bharath Kumar and Suryanarayana, 2011). As per earlier literature, there is no scientific investigations found in *D. sepiaria* on phytoconstituents present. In ethnomedicinal point of view as described above, the GC-MS analysis was carried out with methanolic leaf extract of *D. sepiaria* to investigate the chemical constituents present in it.

2. MATERIALS AND METHODS

2.1. Collection of plant materials and preparation of the extract

The fresh leaves of *D. sepiaria* was collected from the sacred grove of Silambur (Lat, 11.35 °N; Long, 79.31°E), Ariyalur District, Tamil Nadu, India. The specimen was botanically identified and confirmed by Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The preserved plant specimens were submitted to the Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu for further reference. The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The powdered leaf were then subjected to successive extraction with organic solvents such as hexane chloroform and ethanol by Soxhlet method (Catherine *et al.,* 1997). The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed and stored at 4˚C. They were used for GC-MS analysis.

2.2. Preparation of extract

The powdered leaf of *D. sepiaria* (500 g) was extracted with methanol (95%) and double distilled water separately in a soxhlet extractor. The extract was evaporated to dryness at 60°C under reduced pressure in a rotary evaporator and kept in refrigerator at 4°C till used. The extracts were

dissolved in dimethylsulphoxide to make the final concentrations at the time study.

2.3. Preliminary phytochemical screening

A small portion of the dry extracts were subjected to preliminary phytochemical screening to detect the presence of various phytoconstituents present in the leaves of *D. sepiaria* (Harborne, 1973; Evans, 2003).

2.4. Gas chromatography- mass spectrometry (GC-MS) analysis

GC-MS analysis was performed with GC-MS Clarus 500 Perkin Elmer Equipment. Compounds were separated on Elite-5 capillary column (Crossbond 5% Phenyl 95% dimethylpolysiloxane) Oven temperature was programmed as follows: isothermal temperature at 60°C then increased to 200°C at the rate of 10°C/min., then increased up to 280°C at the rate of 5°C/min. held for 9 min. Ionization of the sample components was performed in the Electron energy (70 eV). The helium was used as gas carrier (1ml/min.), and 1.0 µL of sample was injected. The detector was Mass detector Turbomass gold Perkin Elmer. The total running time for GC was 36 min. and software Turbomass 5.2.0 was used in this GC-MS study (Manjamalai *et al.,* 2010).

2.5. Identification of compounds

All the compounds were identified from methanol extracts based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database, NIST (Version year 2005).

3. RESULT AND DISCUSSION

Preliminary phytochemical analysis showed (Table 34) the presence of alkaloids, flavonoids, saponins, and phenols showed in petroleum ether leaf extract and presence of flavonoids, saponins, phenols, steroids in methanolic solvent. Steroids, flavonoids, saponins and phenol Hexane leaf extract showed terpenoids and glycosides only. In chloroform leaf extract showed steroids, tannins, and saponins only.

Table 1. Preliminary phytochemical screening of *Drypetes sepiaria* leaves.

S.No.	Phytochemicals	PE	Chl	AC	E
1.	Alkaloids	+	-	+	-
2.	Steroids	-	+	+	+
3.	Terpenoids	-	-	-	-
4.	Flavonoids	+	-	+	+
5.	Tannins	-	+	-	-
6.	Saponins	+	+	+	+
7.	Glycosides	-	-	-	-
8.	Total phenol	+	-	+	+

PE- Petroleum ether, Chl-Chloroform, AC-Acetone, E-Ethanol, + present, - absent

The chemical constituents identified by the GC-MS analysis on methanolic leaf extract of *D. sepiaria* were enumerated along with Molecular Formula (MF), Molecular Weight (MW), Retention Time (RT), and peak area and peak area (%) is presented in Table-2.

Table 2. Compounds identified in methanolic leaf extract of *Drypetes sepiaria.*

S.No.	Peak Name	Retention Time (min)	Peak Area	% Peak area
1	Name: Propanoic acid, 2-oxo-, methyl ester Formula: $C_4H_6O_3$ MW: 102	2.83	352644	2.8966
2	Name: 2-Furanmethanol Formula: $C_5H_6O_2$ MW: 98	3.69	526502	0.4325
3	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	4.82	251654	2.0671
4	Name: Benzaldehyde Formula: C_7H_6O MW:106	5.46	253243	2.0801
5	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	5.69	232379	1.9087
6	Name: 2-Hydroxy-gamma-butyrolactone Formula: $C_4H_6O_3$ MW: 102	6.25	174726	1.4352

7	Name: 3-Acetylthymine Formula: $C_7H_8N_2O_3$ MW: 168	8.2	341872	2.8081
8	Name: Pyrimidine-4,6-diol, 5-methyl- Formula: $C_5H_6N_2O_2$ MW: 126	8.73	621868	0.5108
9	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	9.53	284421	2.3362
10	Name: 2-Propenal, 3-(2-furanyl)- Formula: $C_7H_6O_2$ MW: 122	10.04	247028	2.0291
11	Name: 2(1H)-Pyrimidinethione, 4,6-diamino- Formula: $C_4H_6N_4S$ MW: 142	10.52	546851	0.4492
12	Name: 1,6;3,4-Dianhydro-2-O-acetyl-á-d-allopyranose Formula: $C_8H_{10}O_5$ MW: 186	10.9	96626	0.0794
13	Name: Dianhydromannitol Formula: $C_6H_{10}O_4$ MW: 146	11.01	318631	2.6172
14	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: $C_6H_6O_3$ MW: 126	11.59	284490	2.3368
15	Name: 2H-Pyran-5-carboxylic acid, 2-oxo-, methyl ester Formula: $C_7H_6O_4$ MW: 154	12.64	544630	0.4474
16	Name: ; Ethyl N-hydroxyacetimidate Formula: $C_4H_9NO_2$ MW: 103	12.95	218384	0.1794
17	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW:150	13.32	950045	7.8036
18	Name: 5-Formylsalicylaldehyde Formula: C8H6O3 MW: 150	13.79	644626	0.5295
19	Name: Phenol, 3,4-dimethoxy- Formula: $C_8H_{10}O_3$ MW: 154	14.15	505296	0.415
20	Name: Benzoic acid, 4-formyl-, methyl ester Formula: $C_9H_8O_3$ MW: 164	14.45	513228	4.2156
21	Name: Benzeneethanol, 4-hydroxy- Formula: $C_8H_{10}O_2$ MW: 138	16.39	405797	3.3332
22	Name: Phenol, 2-methoxy-4-(1-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 138	16.7	346247	2.844
23	Name: Cyclohexane, 1-methylene-4-(1-methylethenyl)- Formula: $C_{10}H_{16}$ MW: 164	18.18	655066	5.3806

24	Name: 2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)- Formula: $C_{10}H_{12}O_3$ MW: 180	18.68	689038	0.566
25	Name: 3',5'-Dimethoxyacetophenone Formula: $C_{10}H_{12}O_3$ MW: 180	19.6	116518	0.9571
26	Name: Benzeneacetic acid, 3,4-dihydroxy- Formula: $C_8H_8O_4$ MW: 168	23.49	151320	12.4293
27	Name: Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester Formula: $C_{10}H_{12}O_4$ MW: 196	24.19	120783	0.9921
28	Name: (R)-(-)-4,4a,5,6,7,8-Hexahydro-4a-methyl-2(3H)-naphthalenone Formula: $C_2H_6N_2O$ MW: 164	24.42	197221	1.62
29	Name: 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol Formula: $C_3H_6N_2O_2$ MW: 180	24.62	432379	3.5515
30	Name: Benzoic acid, 3-formyl-4,6-dihydroxy-2,5-dimethyl-, methyl ester Formula: $C_2H_6N_2O$ MW:224	25.57	144845	1.1897
31	Name: 3,4-Dihydrocoumarin-7-ol Formula:$C_5H_6O_2$ MW: 164	25.82	940934	7.7287
32	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_4H_6O_3$ MW: 296	26.53	531514	4.3658
33	Name: Undecanoic acid, 2-methyl- Formula: $C_8H_{16}O$ MW: 200	28.79	111426	0.9152
34	Name: n-Hexadecanoic acid Formula: $C_7H_{14}O$ MW: 256	30.13	161403	13.2575
35	Name: 9,12-Octadecadienoic acid, methyl ester Formula: $C_6H_8O_3$ MW: 294	32.86	154532	1.2693
36	Name: 10-Octadecenoic acid, methyl ester Formula: C_8H_8O MW: 296	32.99	246271	2.0228

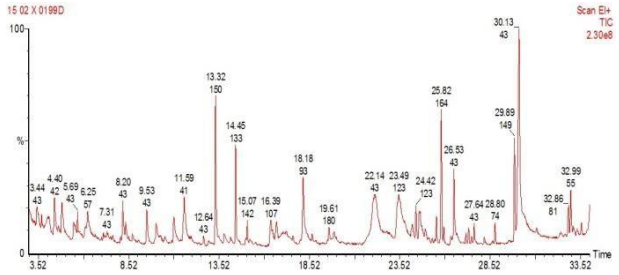

Fig. 1. GC-MS analysis of methanolic leaf extract of *Drypetes sepiaria*

In the methanolic leaf extract of *D. sepiaria,* a total of 36 compounds were identified, of which n-Hexadecanoic acid (13.25%), was found as major compound followed by other compounds namely, Benzeneacetic acid, 3,4-dihydroxy-(12.92%), 2-Methoxy-4-vinylphenol (7.80%), 3,4-Dihydrocoumarin-7-ol (7.72%), and Cyclohexane, 1-methylene-4-(1-methylethenyl)- (5.38%).

Phenolic compounds have antimicrobial properties. Phenol and phenolic compounds have

been extensively used in disinfections. Thus the reported antimicrobial properties of both plants may be attributed to phenolic compounds. Plants with tannins are used for healing of wounds, varicose ulcers and burns (Nafiu *et al.,* 2011). Among the identified phytochemicals, n-hexadecanoic acid has the property of antioxidant activity and it justifies with the earlier work in *Alstonea venenata* (Sutha, 2012).

4. CONCLUSION

The present investigation through the present study revealed that the species *D. sepiaria* is a reliable source of bioactive compounds like fatty acid esters, alcohols, hydrocarbons, alkanes, amines, terpenes, and sugars that justify the traditional usage of this species by the local healers in Tamil Nadu, India, for various ailments. As GC-MS is the first step towards understanding the nature of active principles. Further investigation in this species is suggested for the development of novel drugs.

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